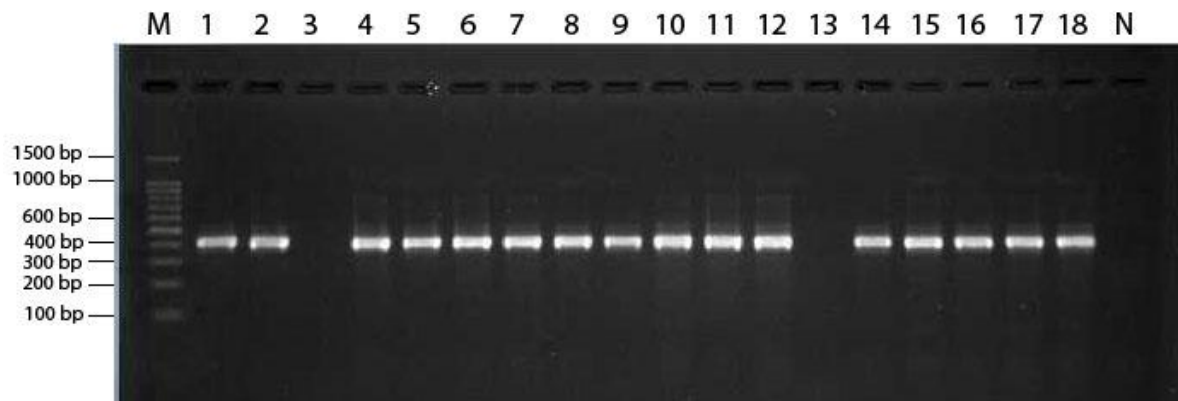


## CHAPTER 4: RESULTS

### 4.1 Confirmation of the *S. Typhimurium* by PCR

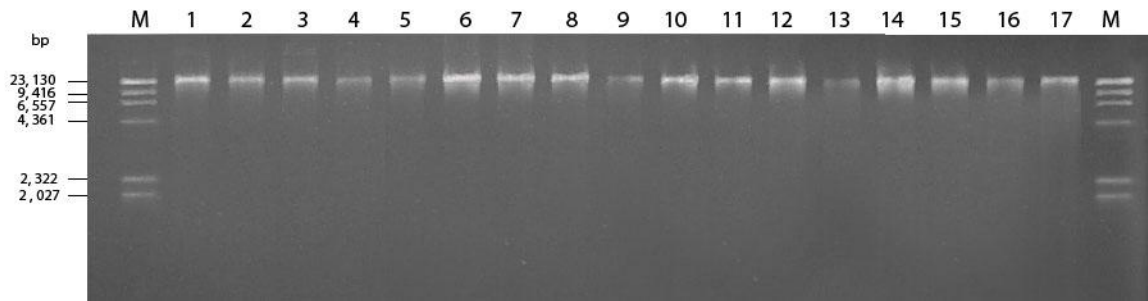
In total 55 *Salmonella* Typhimurium were isolated from sporadic cases, including 14 strains from human and 31 strains from animals. These strains were reported as *Salmonella* Typhimurium based on serological tests provided by the Laboratories. After analyzing of the isolates by using monoplex PCR, the result showed that 49 (90%) strains were indeed *Salmonella* Typhimurium (14 strains from humans and 35 from animals) as indicated by amplicon of TYPH with approximate size of 401 bp (Figure 4.1). Six strains that did not show the characteristic 401bp were not included in further analysis.



**Figure 4.1:** Monoplex-PCR for confirmation of selected *Salmonella* Typhimurium strains by using primers TYPH (401 bp). M: 100bp marker, lane 1: STM 2593/04, lane 2: STM 1234/04, lane 3: *Salmonella* spp., lane 4: STM 2560/05, lane 5: STM 4524/05, lane 6: STM 3000/05, lane 7: STM 01/06, lane 8: STM 3215/03, lane 9: STM 0287/69, lane 10: STM 5231/05, lane 11: STM 196/05, lane 12: STM 6344/95, lane 13: Non STM, lane 14: STM 4307/05, lane 15: STM 2625/05, lane 16: STM 402/05, lane 17: STM 6324/03, lane 18: STM 110187/70, N: Negative control

## 4.2 Extracted genomic DNA

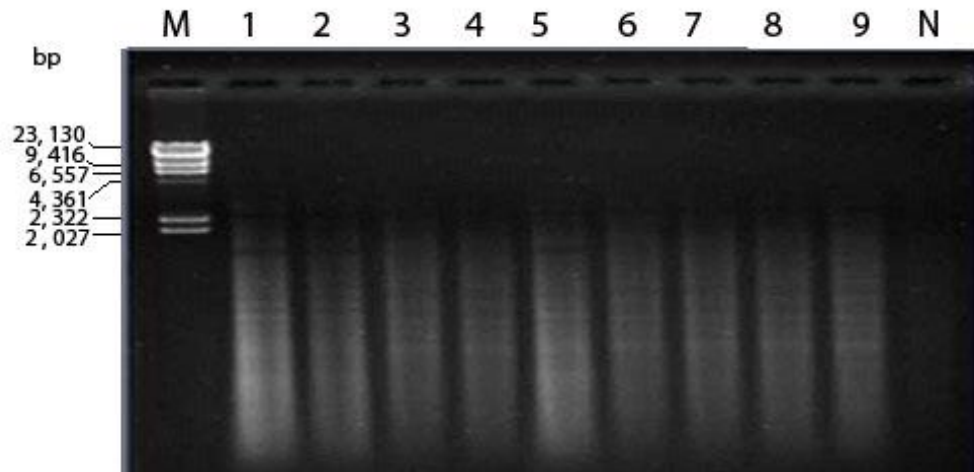
The extracted genomic DNA of 49 isolates of *Salmonella* Typhimurium were analyzed by gel electrophoresis. High quality DNA obtained as indicated by Figure 4.2.



**Figure 4.2:** Extracted genomic DNA of 17 *S. Typhimurium* strains. M: Lambda marker, lane 1: STM 95893/70, lane 2: STM 1621/05, lane 3: STM 8322/03, lane 4: STM 447/05, lane 5: STM 402/05, lane 6: STM 32/05, lane 7: STM 01/06 , lane 8: STM 2555/05, lane 9: STM 196/05, lane 10: STM 447/05, lane 11: STM 3079/05, lane 12: STM 4307/05 (F), lane 13: STM 1204/05, lane 14: STM 6324/03 , lane 15: STM 3866/05, lane 16: STM 2599/05 , lane 17: STM 3503/05, M: Lambda marker

### 4.3 Restricted genomic DNA

All 49 genomic DNA of *Salmonella* Typhimurium isolates were digested by using *Hae*III restriction enzyme. All the DNAs could be digested completely. Example of gel electrophoresis of digested genomic DNA of 9 *S. Typhimurium* shows in Figure 4.3.



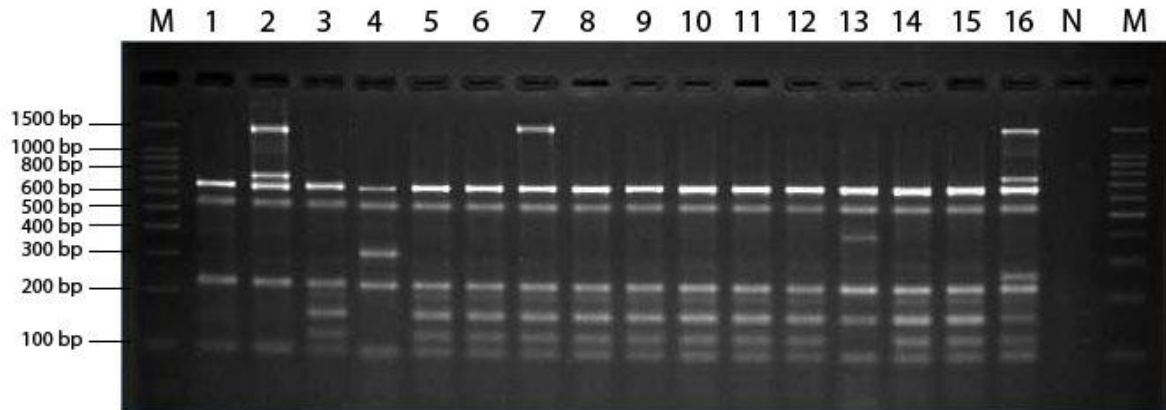
**Figure 4.3: Digested genomics DNA of 9 *S. Typhimurium* strains.** M: Lambda marker, lane 1: STM 1234/04, lane 2: STM 110187/70, lane 3: STM 01/06, lane 4: STM 2599/05, lane 5: STM 32/05, lane 6: STM 5553/04, lane 7: STM 8322/03, lane 8: STM 254/98, lane 9: STM 3000/05, N: Negative Control

#### **4.4 Genetic diversity of *Salmonella* Typhimurium by Restricted AP-PCR**

Restricted AP-PCR was performed by using 3 sets of primers on *Hae*III digested products of 49 *S. Typhimurium* isolates to evaluate the utility of these primers. All the strains were typeable and analysis of the amplified products by agarose gel electrophoresis revealed banding patterns. Restricted AP-PCR amplification of *Hae*III digested DNA by using ResAP-PCR I, ResAP-PCR II and ResAP-PCR III primers was able to generate DNA polymorphism among *S. Typhimurium* isolates tested.

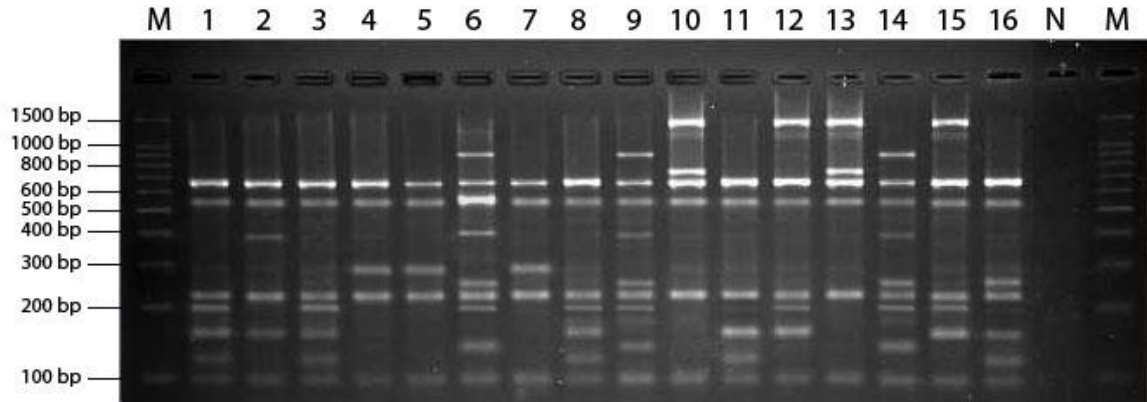
Each strain contained between 3 to 9 bands ranging in size from 100bp to 1500bp. Four conserved bands were seen in *S. Typhimurium* strains in approximate positions of 650bp, 550bp, 230bp and 100 (Figure 4.4, Figure 4.5 and Figure 4.6).

The DNA fingerprinting profiles of the same isolate, generated by Restricted AP-PCR was found to be stable and reproducible on two separate occasions (data are shown in Figure 4.7, Figure 4.8 and Figure 4.9). The intensity of the stained DNA varied for some strains but the same number of the bands and corresponding sizes remained constant. The agarose gels electrophoresis of restricted AP-PCR fragments of representative 49 *S. Typhimurium* are shown in Figure 4.3, Figure 4.4 and Figure 4.5. From these 49 *S. Typhimurium*, 19 different restricted AP-PCR profiles were obtained. Overall, resAP-PCR using these primer combinations has limited value as subtyping tool for *S. Typhimurium* isolates due to the moderate discriminatory power.



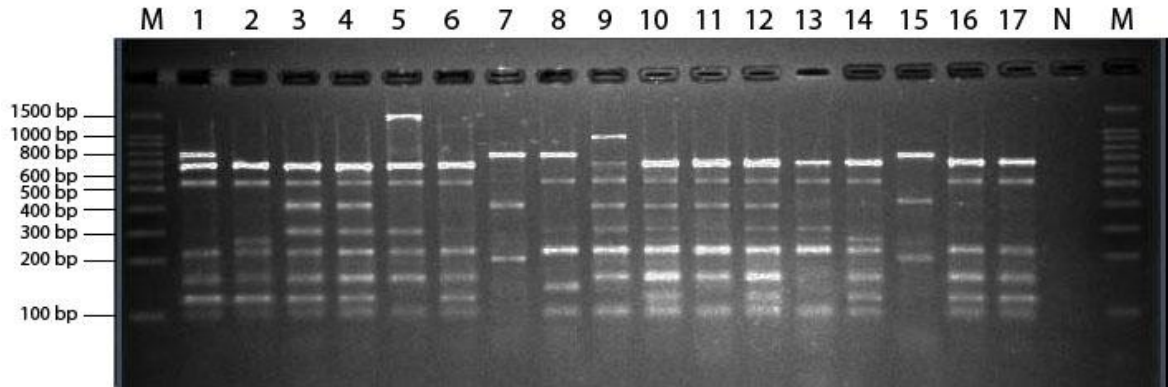
**Figure 4.4:** AP banding patterns for 16 representative *S. enterica* serovar Typhimurium isolates.

M	100bp marker	N	Negative control
Lane 1	STM 981/04	Lane 9	STM 447/05
Lane 2	STM 95893/70	Lane 10	STM 8322/03
Lane 3	STM 3077/05	Lane 11	STM 3000/05
Lane 4	STM 113254/70	Lane 12	STM 5532/03
Lane 5	STM 1621/05	Lane 13	STM 4307/05 (H)
Lane 6	STM 01/06	Lane 14	STM 2560/05
Lane 7	STM 32/05	Lane 15	STM 6324/03
Lane 8	STM 79495/70	Lane 16	STM 0287/69



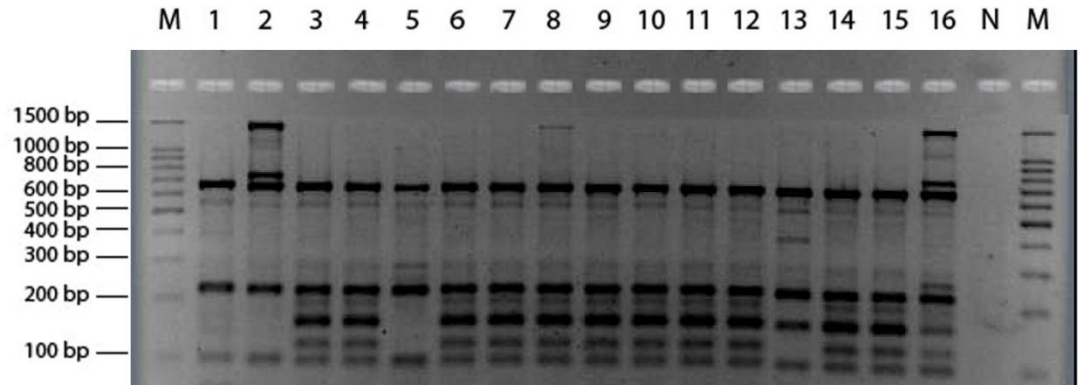
**Figure 4.5:** AP banding patterns for 16 representative *S. enterica* serovar Typhimurium isolates.

M	100bp marker	N	Negative control
Lane 1	STM 402/05	Lane 9	STM 3068/98
Lane 2	STM 4307/05	Lane 10	STM 3503/05
Lane 3	STM 254/98	Lane 11	STM 1377/05
Lane 4	STM 2553/05	Lane 12	STM 3865/05
Lane 5	STM 5553/04	Lane 13	STM 30822/70
Lane 6	STM 2599/05	Lane 14	STM 2559/05
Lane 7	STM 1204/05	Lane 15	STM 6344/95
Lane 8	STM 3866/05	Lane 16	STM 3079/05



**Figure 4.6:** AP banding patterns for 17 representative *S. enterica* serovar Typhimurium isolates.

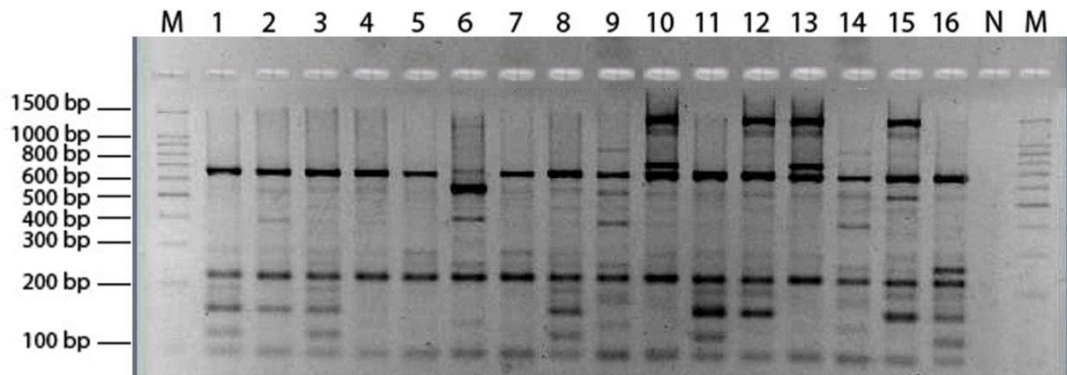
M	100bp marker	N	Negative control
Lane 1	STM 7456/04	Lane 10	STM 2593/04
Lane 2	STM 87098/70	Lane 11	STM 4307/05 (F)
Lane 3	STM 95893/70	Lane 12	STM 110187/70
Lane 4	STM 1234/04	Lane 13	STM 0504/69
Lane 5	STM 4307/05 (G)	Lane 14	STM 5231/05
Lane 6	STM 5229/05	Lane 15	STM 3215/03
Lane 7	STM 287232/77	Lane 16	STM 4524/05
Lane 8	STM 196/05	Lane 17	STM 2555/05
Lane 9	STM 2625/05		



**Figure 4.7:** AP banding patterns for 16 representative *S. enterica* serovar Typhimurium isolates.

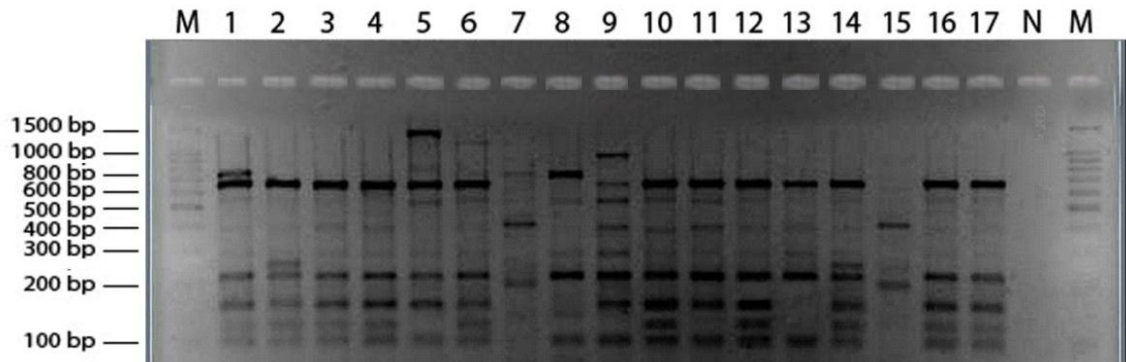
M	100bp marker	N	Negative control
Lane 1	STM 981/04	Lane 9	STM 447/05
Lane 2	STM 95893/70	Lane 10	STM 8322/03
Lane 3	STM 3077/05	Lane 11	STM 3000/05
Lane 4	STM 113254/70	Lane 12	STM 5532/03
Lane 5	STM 1621/05	Lane 13	STM 4307/05 (H)
Lane 6	STM 01/06	Lane 14	STM 2560/05
Lane 7	STM 32/05	Lane 15	STM 6324/03
Lane 8	STM 79495/70	Lane 16	STM 0287/69





**Figure 4.8:** AP banding patterns for 16 representative *S. enterica* serovar Typhimurium isolates.

M	100bp marker	N	Negative control
Lane 1	STM 402/05	Lane 9	STM 3068/98
Lane 2	STM 4307/05	Lane 10	STM 3503/05
Lane 3	STM 254/98	Lane 11	STM 1377/05
Lane 4	STM 2553/05	Lane 12	STM 3865/05
Lane 5	STM 5553/04	Lane 13	STM 30822/70
Lane 6	STM 2599/05	Lane 14	STM 2559/05
Lane 7	STM 1204/05	Lane 15	STM 6344/95
Lane 8	STM 3866/05	Lane 16	STM 3079/05



**Figure 4.9:** AP banding patterns for 17 representative *S. enterica* serovar Typhimurium isolates.

M	100bp marker	N	Negative control
Lane 1	STM 7456/04	Lane 10	STM 2593/04
Lane 2	STM 87098/70	Lane 11	STM 4307/05 (F)
Lane 3	STM 95893/70	Lane 12	STM 110187/70
Lane 4	STM 1234/04	Lane 13	STM 0504/69
Lane 5	STM 4307/05 (G)	Lane 14	STM 5231/05
Lane 6	STM 5229/05	Lane 15	STM 3215/03
Lane 7	STM 287232/77	Lane 16	STM 4524/05
Lane 8	STM 196/05	Lane 17	STM 2555/05
Lane 9	STM 2625/05		

The dendrogram of all the 49 *S. Typhimurium* strains was constructed using a clustering algorithm of the unweighted pair group arithmetic means method (UPGMA). Based on 70% similarity 2 major clusters with total of 19 banding profile patterns were generated (Figure 4.10).

Among the 19 AP profiles, AP3 profile was the predominant profile and was represented by 14 strains (28%), followed by AP1, represented by 4 strains (8%). Five AP profiles (AP18, AP10, AP13, AP 17 and AP2) were present in 3 strains (6%) and four AP profiles (AP5, AP8, AP11 and AP15) were presented in 2 strains (4%). The rest of 8 profiles were presented in unique strain for each profile. All the profiles are shown in Table 4.1.

Cluster A is a major cluster comprising of two subcluters, A1 and A2. Subcluster A1 is contained 24 strains. It is included zoonotic ( $n=18$ ), one human ( $n=6$ ) and 58% of them were isolated in year 2005. From 18 zoonotic strains, chicken strains were the majority with  $n= 9$  where 66% of them were isolated in year 2005. It is the largest subcluster and 7 AP profiles were observed in this subcluster. The dominant profile in this subcluster was AP3 profile which contained 14 strains, chicken ( $n=6$ ), human ( $n=3$ ), fish ( $n=1$ ), frog ( $n=1$ ), cattle ( $n=1$ ), swine ( $n=1$ ) and animal ( $n=1$ ). AP10 was defined for 3 strains (STM 3079/05=cattle, STM 5231/05=cattle and STM 87098/70=human. AP13 profile was presented by three strains from chicken (STM 402/05, STM 254/98 and STM 3866/05). This subcluster consisted of four isolates having apparently unique AP profiles (AP7, AP9, AP6 and AP4) (Figure 4.10).

**Table 4.1:** 49 *S.Typhimurium* isolates from sporadic sources which represent 19 AP profiles

No	Strain Numbers	Source	Restricted AP-PCR Patterns	Number of bands
1	STM 110187/70	Human	AP 1	8
2	STM 02/06	Human	AP 1	8
3	STM 2593/04	Cattle	AP 1	8
4	STM 1234/04	Animal	AP 1	8
5	STM 95893/70	Human	AP 2	6
6	STM 30822/70	Human	AP 2	6
7	STM 3503/05	Chicken	AP 2	6
8	STM 01/06	Human	AP 3	6
9	STM 79495/70	Human	AP 3	6
10	STM 447/05	Human	AP 3	6
11	STM 3077/05	Swine	AP 3	6
12	STM 1621/05	Chicken	AP 3	6
13	STM 8322/03	Chicken	AP 3	6
14	STM 2560/05	Chicken	AP 3	6
15	STM 6324/03	Chicken	AP 3	6
16	STM 4524/05	Chicken	AP 3	6
17	STM 5229/05	Chicken	AP 3	6
18	STM 2555/05	Cattle	AP 3	6
19	STM 3000/05	Fish	AP 3	6
20	STM 1377/05	Frog	AP 3	6
21	STM 5532/04	Animal	AP 3	6
22	STM 0287/69	Human	AP 4	9
23	STM 2553/05	Cattle	AP 5	4
24	STM 5553/04	Animal	AP 5	4
25	STM 981/04	Animal	AP 6	5
26	STM 32/05	Human	AP 7	7
27	STM 6344/95	Animal	AP 8	7
28	STM 3865/05	Chicken	AP 8	7
29	STM 7456/04	Cattle	AP 9	7
30	STM 3079/05	Cattle	AP 10	7
31	STM 5231/05	Cattle	AP 10	7
32	STM 87098/70	Human	AP 10	7
33	STM 287232/77	Human	AP 11	3
34	STM 3215/03	Animal	AP 11	3
35	STM 196/05	Human	AP 12	6
36	STM 402/05	Chicken	AP 13	7
37	STM 254/98	Chicken	AP 13	7
38	STM 3866/05	Chicken	AP 13	7
39	STM 2625/05	Chicken	AP 14	8
40	STM 3068/98	Chicken	AP 15	9
41	STM 2559/05	Cattle	AP 15	9
42	STM 43/7/05 (G)	Chicken	AP 16	7
43	STM 43/7/05	Chicken	AP 17	6
44	STM 43/7/05 (F)	Chicken	AP 17	6
45	STM 43/7/05 (H)	Chicken	AP 17	6
46	STM 113254/70	Human	AP 18	5
47	STM 0504/69	Human	AP 18	5
48	STM 1204/05	Chicken	AP 18	5
49	STM 2599/05	Animal	AP 19	9

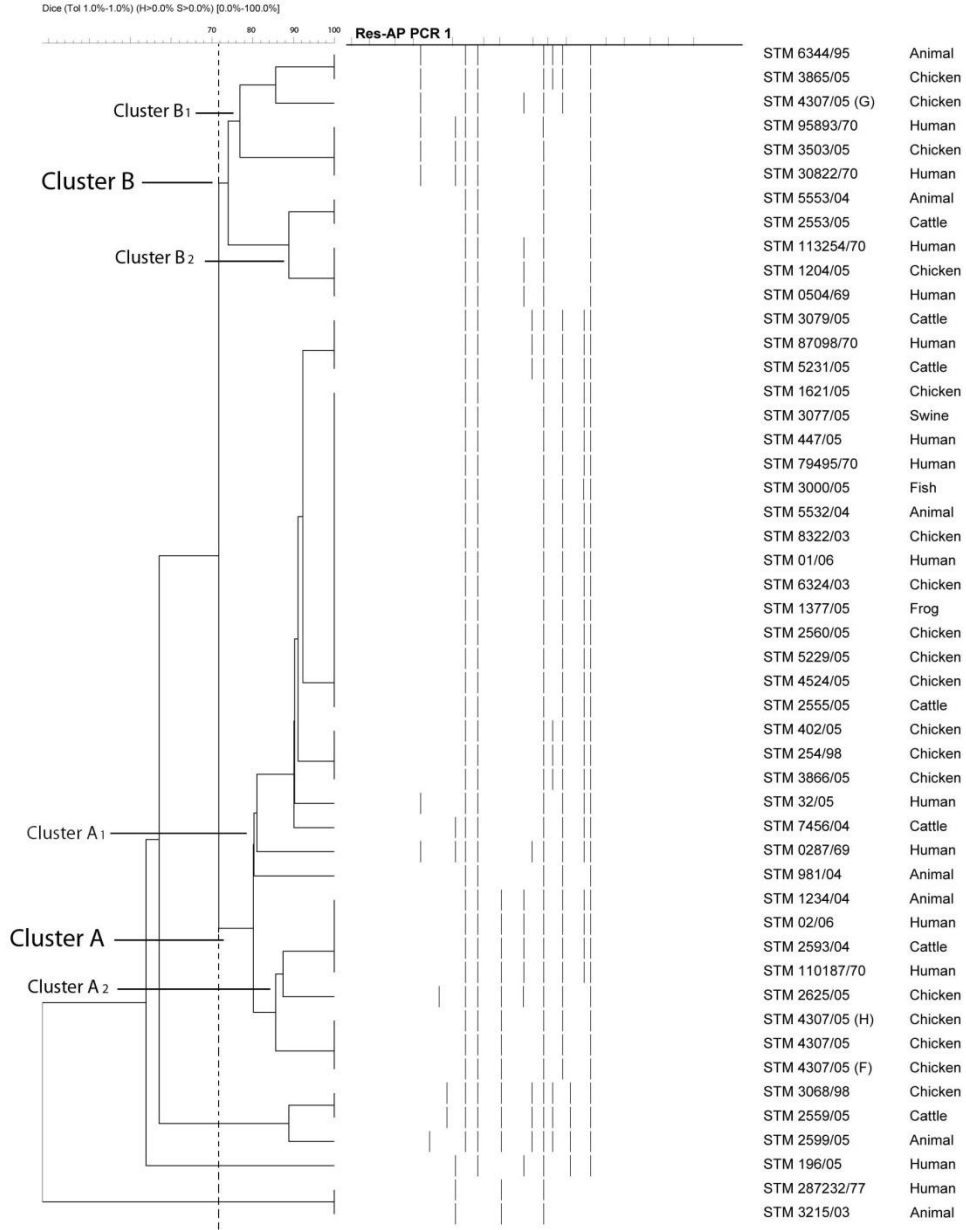
Subcluster A2 contained 6 zoonotic strains (chicken=4, cattle=1, animal=1) and 2 human strains which isolated between 1970 until 2006. In this subcluster, AP 1 profile was presented in 4 strains (STM 110187/70=human, STM 02/06=human, STM 2593=cattle and STM 1234/04=animal). Also, AP 17 profile was observed in three strains (STM 4307/05=chicken, STM 4307/05 (H) =chicken and 4307/05 (F) =chicken). AP14 was represented by one strain (STM 2625/05=chicken) (Figure 4.10).

Cluster B comprised two subclusters, B1 and B2. Subcluster B1 consisted of 6 strains, 3 from chicken (isolated in year 2005), 2 from humans (isolated in year 1970), and 1 from zoonotic. Hence, there were 3 AP profiles in this cluster. The AP2 which present in 3 strains (STM 95893/70= human, STM 30822/70= human, STM 3503/05= chicken) was the majority of all profiles. AP8 profile was observed in two strains (STM 3865/05 = chicken, STM 6344/95= animal). While, AP16 profile was observed in one strain (STM 4307/05 (G) =chicken). All chicken strains in this subcluster were isolated in year 2005 (Figure 4.10).

Subcluster B2 consisted of 5 strains comprising two AP profiles. AP18 was presented in 3 strains (STM 113254/70= human, STM 0504/69=human, STM 1204/05= chicken). AP5 was observed in two strains (STM 5553/04=animal and STM 2553/04=cattle) (Figure 4.10).

The rest of AP profiles such as, AP15, AP19, AP12, AP11, were not included to any cluster. AP15 which represented by two strains (STM 3068/98=chicken and STM 2559/05=cattle). AP11 is the most diverse profile which represented by two strains

(STM 287232/77=human and STM 3215=animal). AP12 (STM 196/05=human) and AP19 (STM 2599/05=animal) were observed in only one strain (Figure 4.10).



**Figure 4.10:** Dendrogram (Left panel) and DNA fingerprints (Right panel) generated by GelCompare II software. It showed the relationship of representing restricted AP fingerprints for 49 *S. Typhimurium* isolates in Malaysia. Pattern clustering on a matrix of dice coefficient was based on the unweighted pair group method with arithmetic averages (UPGMA).